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***Sarcocystis campestris* From Naturally Infected 13-Lined Ground Squirrels, *Spermophilus tridecemlineatus tridecemlineatus*, From Nebraska**

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ABSTRACT: Grossly visible sarcocysts were seen in the skeletal muscles of 1 of 12 13-lined ground squirrels, *Spermophilus tridecemlineatus tridecemlineatus*, collected in Nebraska. The tissue cyst wall was up to 5.0 µm thick and contained spikelike projections. Transmission electron microscopy of tissue cysts revealed they were similar to *Sarcocystis campestris* Cawthorn, Wobeser, and Gajadhar, 1983, previously known only from experimental infections in Richardson's ground squirrel *Spermophilus richardsonii*. Prominent electron-dense bodies were observed lining the microfilaments present in the spikelike projections of the sarcocyst wall. This is the first report of *S. campestris* in a natural intermediate host and the first report of this parasite outside of Saskatoon, Canada.

Cawthorn et al. (1983) described *Sarcocystis campestris* from Richardson's ground squirrels *Spermophilus richardsonii*, fed sporocysts collected from naturally infected badgers, *Taxidea taxus*. They reported that sarcocysts became grossly visible by day 258 postinoculation. Sporocysts were found in badgers that were fed muscle from the experimentally infected ground squirrels, demonstrating that the life cycle consisted of a badger definitive host and a ground squirrel intermediate host. Wobeser et al. (1983) described the pathogenicity of *S. campestris* sporocysts for Richardson's ground squirrels. The natural intermediate host for *S. campestris* is not known.

We observed grossly visible sarcocysts in 13-lined ground squirrels, *Spermophilus tridecemlineatus tridecemlineatus*

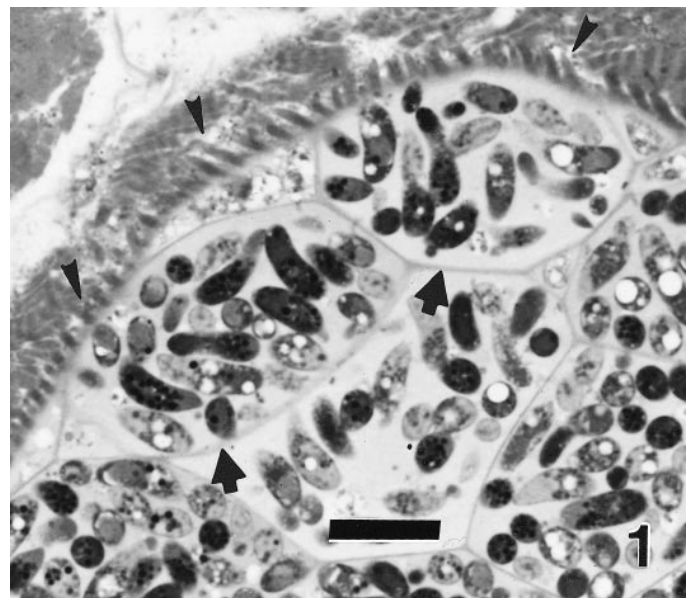
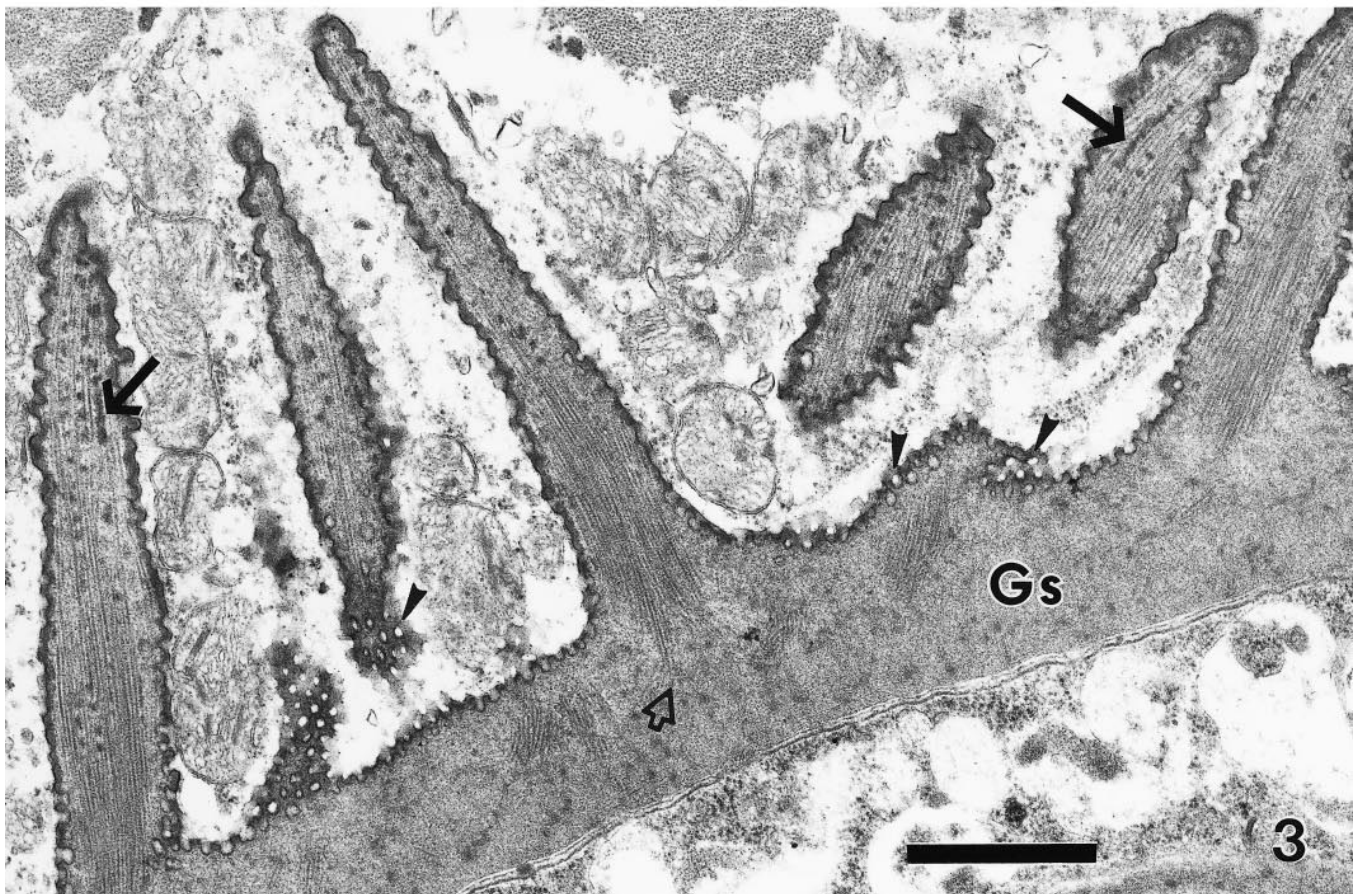
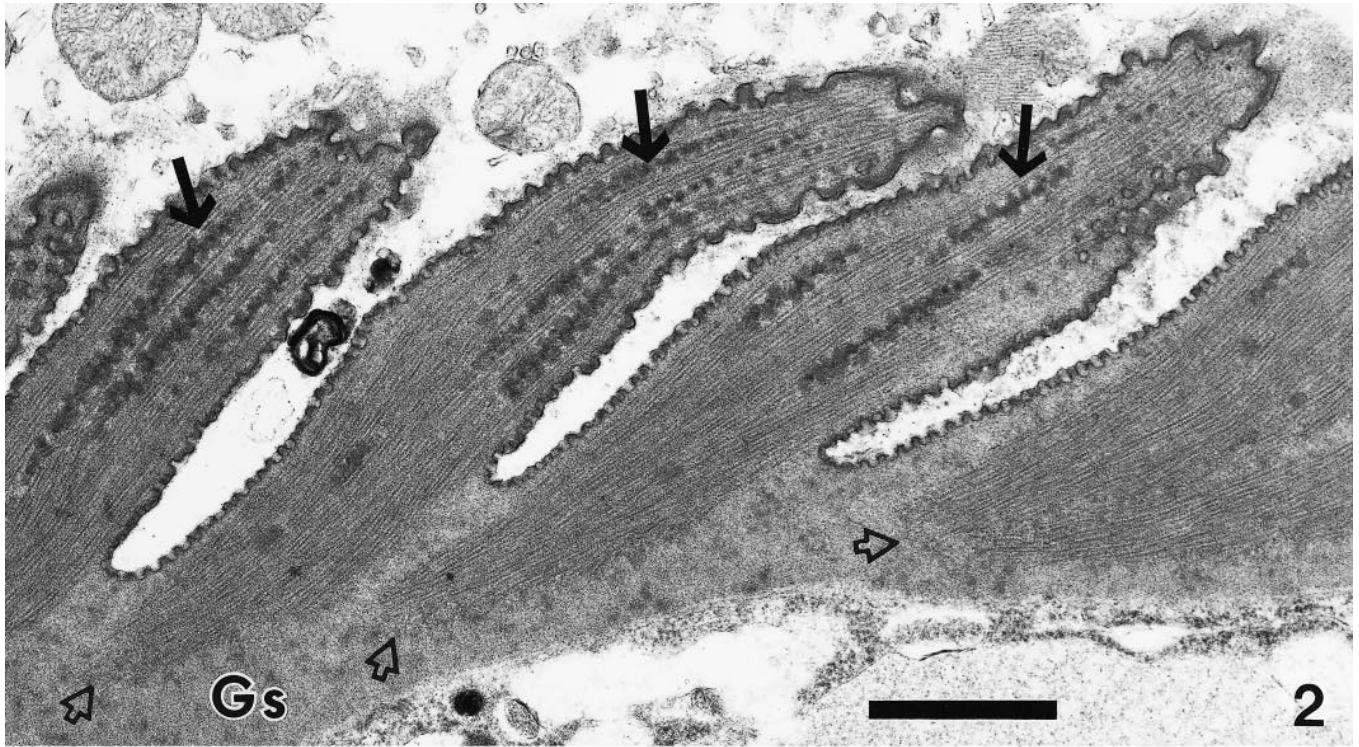


FIGURE 1. Photomicrograph of a sarcocyst of *Sarcocystis campestris* in a 13-lined ground squirrel. Note the spikelike projections (arrowheads) on the sarcocyst wall. The septa (arrows) divide the sarcocyst into compartments. Toluidine blue stain. Bar = 10 µm.



FIGURES 2, 3. Transmission electron photomicrographs of sarcocysts of *Sarcocystis campestris* in a 13-lined ground squirrel. **2.** Spikelike projections containing rows of electron-dense bodies (arrows) along the microfilaments. Note that the microfilaments (open arrows) extend into the ground substance (GS). Bar = 1 μ m. **3.** Note the ornamentalations on the primary cyst wall and that holes (arrowheads) in the primary cyst wall are due to the plane of sectioning. Spikelike projections containing rows of electron-dense bodies (arrows) along the microfilaments are present and microfilaments (open arrows) extend into the ground substance (GS). Bar = 1 μ m.

(Mitchill), from Nebraska. We did not examine tissues for microscopic sarcocysts. The present report documents the occurrence of *S. campestris* in 13-lined ground squirrels that serve as a natural intermediate host and provides additional information on the structure of the sarcocyst of this species.

Portions of internal and external abdominal oblique muscles and internal intercostal muscles with grossly visible sarcocysts were fixed in 2% (v/v) glutaraldehyde in phosphate buffer or 0.4% glutaraldehyde/4% formalin and mailed to the Center for Molecular Medicine and Infectious Diseases, Virginia–Maryland Regional College of Veterinary Medicine, Virginia Tech, Blacksburg, Virginia for processing and ultrastructural examinations. Tissues were postfixed in 1% (w/v) osmium tetroxide, dehydrated in a series of ethanols, passed through 2 changes of propylene oxide, and embedded in Poly/Bed 812 resin (Polysciences Inc., Warrington, Pennsylvania). Thin sections were stained with uranyl acetate and lead citrate and examined with a JEOL-100 CX II transmission electron microscope (TEM) operating at 80 kV. Additionally, 1- μ m-thick sections were placed on glass microscope slides and stained with toluidine blue and examined by light microscopy. Portions of muscles were also fixed in 10% neutral buffered formalin solution and processed for light microscopic examination after staining with hematoxylin and eosin (H&E).

One of 12 13-lined ground squirrels collected from Adams (n = 2), Clay (n = 8), and Fillmore (n = 2) counties had grossly visible sarcocysts. Nine of the animals were male and 3 were females. The ages were not determined.

Examination of grossly visible sarcocysts in histological sections stained with toluidine blue or H&E were similar (Fig. 1). The cyst wall was 1–2 μ m thick and contained spikelike projections that were 0.5–5.0 μ m long depending on the plane of section. Bradyzoites were 10.0–12.0 by 2.0–3.0 μ m in size. Toluidine blue- and H&E-stained tissue sections have been deposited in the United States National Parasite Collection (USNPC), Beltsville, Maryland, USNPC no. 089747.00.

Transmission electron microscopic examination of 3 sarcocysts from the naturally infected 13-lined ground squirrel were similar (Figs. 2, 3). The primary cyst wall was composed of the parasitophorous vacuole (PV) membrane and underlying electron-dense material. The PV membrane was ornamented with bumplike or knoblike structures that were electron dense in the upper portions but composed only of a small portion of the PV membrane at the lower portion. When sectioned appropriately, the thinner portions of the electron-dense ornamentations appeared as holes in the primary sarcocyst wall (Fig. 3). There was a granular layer immediately beneath the primary sarcocyst wall, and it gave rise to the ground substance and septa that divided the sarcocyst into compartments (Fig. 1).

Metrocytes were visible at the periphery of the sarcocyst. The sarcocyst wall contained numerous spikelike projections. The core of the spikelike projections contained bundles of microfilaments that extended from the anterior portion to $\frac{1}{2}$ to $\frac{3}{4}$ the depth of the ground substance. Electron-dense bodies were observed in rows along groups of these microfilaments (Figs. 2, 3). These rows of electron-dense bodies were up to $\frac{3}{4}$ the length of a spikelike projection (Fig. 2) or in small clusters in other spikelike projections (Fig. 3) in sarcocysts that were appropriately sectioned. Bradyzoites contained all the organelles typical of this stage (Dubey et al., 1989).

None of 5 badgers collected from Adams (2), Clay (2), or Fillmore (1) counties had detectable sporocysts in their fecal samples after flotation in Sheather's sugar solution.

Cawthorn et al. (1983) only briefly described the ultrastructure of sarcocysts of *S. campestris* and their TEM photomicrograph is of a 113-day-old sarcocyst. They mentioned that a central core containing longitudinal striations was present in the projections of the sarcocyst wall. We interpret these to be the microfilaments observed in the spikelike projections in the present study. They did not mention the occurrence of rows of electron-dense bodies in the projections of the sarcocyst wall in their study. It is not clear if Cawthorn et al. (1983) examined sarcocysts that were older than 113 days. Our sarcocysts were grossly visible, and it may be that they were older than the sarcocysts studied by Cawthorn et al. (1983). The differences in ultrastructure may be due to age of the sarcocyst (Dubey et al., 1989). However, several TEM photomicrographs of sarcocysts of *S. campestris* illustrated by Dubey et al. (1989) contain electron-dense bodies that are associated with microfilaments present in the spikelike projections of the sarcocyst wall. The source of the Dubey et al. (1989) material was the study of Cawthorn et al. (1983). It appears that the electron-dense bodies were present in the sarcocysts of *S. campestris* existing in the experimentally infected Richardson's ground squirrels but were not mentioned in the original species description by Cawthorn et al. (1983).

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